Polymorphism at the *Adh* and *αGpdh* loci in *Drosophila melanogaster*: effects of rearing temperature on developmental rate, body weight, and some biochemical parameters

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The role of developmental time in the world-wide cline of *Adh* and *αGpdh* allele frequencies of *Drosophila melanogaster*, and the relationship with weight and some biochemical characters, were investigated. Experimental strains were constructed with different combinations of *Adh* and *αGpdh* alleles but with similar genetic background. Developmental time, adult weight, protein- and triglyceride-content, and ADH and α GPDH enzyme activity were measured at a rearing temperature of 20, 25 and 29°C. Genotype effects were found in all studied characters. In general the developmental times of genotypes were: $Adh^{FF} < Adh^{FS} < Adh^{SS}$ and $\alpha Gpdh^{FF} > \alpha Gpdh^{FS} = \alpha Gpdh^{SS}$. Developmental time and adult weight were strongly affected by rearing temperature. Triglyceride content and ADH and α GPDH enzyme activity were slightly affected by temperature. Interactions between genotype and temperature effects were found for developmental rate, adult weight and protein content. No trade off was observed between developmental time on the one hand and adult weight, protein- and triglyceride-content, and ADH and α GPDH enzyme activity on the other hand. It is argued that developmental rate differences might be one of the underlying mechanisms of the world-wide cline of the *Adh* and *αGpdh* allele frequencies.

Keywords: *Adh*, *αGpdh*, developmental time, *Drosophila melanogaster*, temperature, weight.

Introduction

The alcohol dehydrogenase (*Adh*) and α -glycerophosphate dehydrogenase (*αGpdh*) loci are two of the most extensively studied allozyme systems of *Drosophila melanogaster*. They provide an excellent opportunity to study interactions between physiological effects of gene-enzyme systems. Both loci have two common electrophoretic alleles: *Adh*^F, *Adh*^S, *αGpdh*^F and *αGpdh*^S. Nearly all natural populations are polymorphic for both loci. The frequencies of these alleles show world-wide clinal variation: the frequencies of *Adh*^S and *αGpdh*^F decrease with increasing latitude (Oakeshott *et al.*, 1982). An important question is whether these clines are under the control of natural selection. Correlation analysis of climatic data revealed no consistent significant correlations (Anderson *et al.*, 1987). In the laboratory, stress from alcohol or temperature can cause differential selection of *Adh* and *αGpdh* alleles (e.g. Van Delden, 1982; Zera *et al.*, 1985). Under normal laboratory conditions equi-

librium selection has been demonstrated for *Adh* (Bijlsma-Meeles & Bijlsma, 1988) and *αGpdh* (Palabost-Charles, 1980). Selection at temperatures between 25 and 30°C may lead to an increase of the *Adh*^S and *αGpdh*^F alleles (Alahiotis, 1982; Van Delden, 1984). Vigue *et al.* (1982) and Oakeshott *et al.* (1985), however, found no effect of temperature on *Adh* or *αGpdh* allele frequencies. Van Delden (1984) and Van Delden & Kamping (1989) ascribe the advantage of the *Adh*^S and *αGpdh*^F alleles at a higher temperature, for the greater part, to linkage of both alleles with the inversion *In*(2L)*t*.

Differences in developmental time between genotypes may be an important underlying mechanism of selection. Developmental rate is a quantitative trait in which many biochemical processes play a role (Robertson, 1963). ADH and α GPDH may be two of the enzymes involved: differences in developmental rate between genotypes are described for both *Adh* and *αGpdh* (e.g. Kohane & Parsons, 1986; Barnes *et al.*, 1989; Izquierdo & Rubio, 1989). The fitness interac-

tion between *Adh* and *αGpdh* is described by Cavener & Clegg (1981) who followed *Adh* and *αGpdh* allele frequencies of populations on medium with ethanol. McKechnie & Geer (1988) describe epistatic interactions between *Adh* and *αGpdh* alleles in larval ethanol tolerance. Metabolic interaction between ADH and *αGPDH* is plausible in two biochemical pathways.

1 Both enzymes influence the NADH/NAD⁺ ratio (Cavener, 1983): the oxidation of ethanol by ADH generates NADH, *αGPDH* regenerates NAD⁺ in concert with mitochondrial *αGPO* to form the '*αGPDH*-shuttle' (O'Brien & MacIntyre, 1978).

2 Both enzymes play a role in lipid synthesis (Geer *et al.*, 1983): ethanol, oxidized by ADH, becomes for the greater part incorporated in fatty acids, while *αGPDH* produces glycerophosphate, a precursor of phospholipids and triglycerides.

The present article describes measurements of the rate of development for *Adh αGpdh* genotypes at different pre-adult temperatures under normal laboratory conditions. Because of a possible trade off with developmental time, adult body weight and protein and triglycerides content were measured. ADH and *αGPDH* enzyme activities were measured to investigate the relations with the previous mentioned parameters. The results are discussed in relation to the world-wide clinal variation in *Adh* and *αGpdh* allele frequencies.

Materials and methods

Experimental stocks

Sublines, homozygous for *Adh*^F or *Adh*^S and *αGpdh*^F or *αGpdh*^S, were derived from the polymorphic wild-type laboratory strain Groningen 83. This strain was founded in 1983 with 403 females from a fruit market in Groningen, The Netherlands. At the time the sublines were derived the allele frequencies in the strain were 19 per cent *Adh*^S and 8 per cent *αGpdh*^S. Eight single pairs were selected which contained both *Adh* and both *αGpdh* alleles. From each of these eight pairs a quartet of sublines, each homozygous for one of the four possible combinations of *Adh* and *αGpdh* alleles, was derived by four subsequent single-pair crosses for each of the genotypes. The eight quartets (1–8) were combined into two sets using the same genotypes from the quartets 1–4 (A) and idem for the quartets 5–8 (B). This resulted in two sets (A and B) with a similar genetic background within each set. The strains were checked for the occurrence of the inversion *In(2L)t* by scoring recombination with the mutants dumpy and black (Van Delden & Kamping, 1989); this inversion did not occur. Lines were obtained for the

experiments from crosses between sets A and B, resulting in *Adh αGpdh* genotypes with a similar genetic background (A×B). Nine crosses (and their reciprocals) were performed to obtain all possible *Adh αGpdh* genotypes, see Table 1. For statistical reasons only one double heterozygous genotype was used.

Rearing conditions

Standard medium was prepared according to Mittler & Bennet (1962) with slight modifications: 1000 ml water, 19 g agar, 54 g sucrose, 32 g sterilized dry yeast and 13 ml nipagine solution (10 g nipagine in 100 ml 96 per cent ethanol). All incubators had 24 h light and ~50 per cent RH. To obtain the parental generation, flies from the sets A and B were allowed to lay eggs for 10 h on dishes with fresh medium. The eggs were transferred to 250 ml bottles with 30 ml fresh medium, 200 eggs per bottle, and incubated at 25°C. After eclosion, virgin females and males were collected and the sexes were aged separately for 5 days in vials. The flies were crossed according to the scheme given in Table 1. Reciprocal crosses were kept separately. Flies were stored in bottles, supplied with live yeast. After 3 days flies were allowed to lay eggs for 4 h on dishes with fresh medium and a drop of live yeast. The eggs were washed from the medium (with water of 25°C) and sets of 50 eggs were put in plastic vials of 80×23 mm with 8 ml fresh medium. Twelve hours after egg laying the vials were put at the appropriate temperature (20, 25 or 29°C). Five vials were incubated at each temperature for each cross. There were no differences in survival between the genotypes. The mean survival from egg to adult was 88 per cent.

Table 1 Origin of the experimental lines from the sets A and B. The first and second letter refer to the *Adh* genotype, the third and fourth letter to the *αGpdh* genotype

	Set B			
	<i>Adh</i> ^{FF} <i>αGpdh</i> ^{FF}	<i>Adh</i> ^{FF} <i>αGpdh</i> ^{SS}	<i>Adh</i> ^{SS} <i>αGpdh</i> ^{FF}	<i>Adh</i> ^{SS} <i>αGpdh</i> ^{SS}
Set A				
<i>Adh</i> ^{FF}	FF FF	FF FS		FS FS
<i>αGpdh</i> ^{FF}				
<i>Adh</i> ^{FF}		FF SS		FS SS
<i>αGpdh</i> ^{SS}				
<i>Adh</i> ^{SS}	FS FF		SS FF	
<i>αGpdh</i> ^{FF}				
<i>Adh</i> ^{SS}			SS FS	SS SS
<i>αGpdh</i> ^{SS}				

Developmental time

Flies were removed after eclosion at regular intervals depending on temperature (~8 h at 20°C; ~6 h at 25°C; ~4 h at 29°C), counted and stored in vials at 25°C, for weight determination and biochemical assays. The developmental time of individual flies was taken as the midpoint of the interval. The developmental time per vial was calculated as the average of the developmental times of the individual flies.

Adult weight

The flies were etherized a few hours after collecting, the sexes separated and aged separately for 6 days at 25°C in vials with 20 flies each. On the sixth day the flies were etherized and weighed in groups of 10 (five replicates) to an accuracy of 0.01 mg. Immediately after weighing each group was stored at -80°C for later assay of protein, triglycerides and α GPDH and ADH enzyme activity.

Biochemical assays

Groups of 10 males (five replicates) were homogenized in 2 ml cold homogenization buffer (0.05 M glycine-NaOH, 1 mM EDTA, pH 9.5). A sample of 0.025 ml was taken and stored at -20°C for the assay of triglycerides. The homogenates were centrifuged for 2 min at 11 000 g. To assay protein, 0.05 ml supernate was stored at -20°C. The remaining supernate was stored at 0°C for immediate assay of α GPDH and ADH. Five replicates were assayed in duplo for each type. The final results (see tables) might have less than five replicates, because some replicates were lost during the assays.

Protein. This was assayed according to Bradford (1976), using the Biorad protein assay kit. To 0.05 ml defrosted homogenate, 2.5 ml reagent was added. After 20 min the extinction was measured at 590 nm. The total protein content was calculated in micrograms using BSA as a standard.

Triglycerides. These were assayed using a modification of the enzymatic procedure described by Clark & Gellman (1985). They found a good correlation between enzymatic triglyceride determination and the determination of total lipids by extraction. In the enzymatic procedure triglycerides were hydrolysed and the released glycerol quantified enzymatically (Bucolo & David, 1973). The reagents were obtained from Sigma as Kit 334-UV and consisted of reagents A and B. To the defrosted sample of 0.025 ml homo-

genate, 1.225 ml reagent A was added. This was incubated at 35°C for 5 min, centrifuged for 2 min at 11 000 g, and placed at 30°C for 3 min. After reading OD₅₉₀, 0.025 ml reagent B was added. The OD₅₉₀ was read again after exactly 5 min at 30°C. Triglycerides were calculated in micrograms using a triglyceride standard (Sigma 336-300).

α GPDH enzyme activity. This was measured using a modification of the procedure described by Rechsteiner (1970). To 0.85 ml buffer (0.05 M glycine-NaOH, 1 mM EDTA, pH 9.5) at 30°C, 0.05 ml homogenate was added, followed by 0.1 ml reagent buffer of 30°C. The reagent consisted of glycine-NaOH buffer (see above) containing NAD⁺ and DL- α -glycerophosphate. The final concentration was 5 mM NAD⁺ and 10 mM α -glycerophosphate. The reaction rate was measured after 30 s during 90 s at 30°C and 340 nm (nine readings) in a Labsystems Chemistry Analyser FP401.

ADH enzyme activity. This was measured following the same procedure as for α GPDH. Isopropanol was used instead of α -glycerophosphate, at a final concentration of 200 mM.

Statistical analysis

ANOVA was performed to test the effects of *Adh* genotype, *αGpdh* genotype and temperature. The reciprocal crosses were taken together. Where the ANOVA indicated significant differences, the data are shown in figures. Tukey's test for multiple comparisons was performed on groups of three temperatures or three genotypes with a level of significance of 5 per cent; significant differences are indicated in the figures. In some cases (indicated in the results) data were standardized for temperature or genotype differences by subtracting the mean value per temperature or genotype from each replicate of that particular temperature or genotype.

Results

Only male data were analysed because females are more variable in weight and biochemical traits as there is variation in the number of eggs an individual female is carrying. Furthermore, ANOVA with respect to differences in developmental time between the sexes only showed a main effect of sex, and interaction between sex and temperature: females developed faster than males at all temperatures, although to a varying extent. No interactions were observed between sex and genotype.

Egg to adult developmental time

ANOVA for the effects of *Adh* genotype, *aGpdh* genotype and temperature on developmental time (Table 2) showed significant variation for all main effects and interactions. Developmental time was negatively correlated with rearing temperature (Fig. 1a). Figure 1b and c show the developmental time of *Adh* and *aGpdh* genotypes at different temperatures. Figure 1d and e show the interactions between *Adh* and *aGpdh* genotypes at different temperatures.

At all rearing temperatures *Adh*^{FF} developed significantly faster than *Adh*^{SS}, and *Adh*^{FS} was intermediate (Fig. 1b). This general picture of developmental rate differences between *Adh* genotypes is only slightly affected by interactions with temperature (Fig. 1c) or *aGpdh* genotype (Fig. 1d). Figure 1e shows the interaction of *Adh* genotype with *aGpdh* genotype and temperature: at 29°C *Adh*^{FF} developed significantly faster than *Adh*^{SS} within all three *aGpdh* genotypes, at 25°C only within *aGpdh*^{FS} and *aGpdh*^{SS} were differences between *Adh* genotypes significant, at 20°C only within *aGpdh*^{FS} were significant differences in the developmental time of *Adh* genotypes found. Notwithstanding significant interactions the results show that *Adh*^{FF} develops faster than *Adh*^{SS}, while the heterozygote is intermediate.

aGpdh^{FF} developed more slowly than *aGpdh*^{FS} and *aGpdh*^{SS} (Fig. 1b), although differences were not significant at 29°C (Fig. 1c). Differences in developmental time between *aGpdh* genotypes showed a clear pattern of interaction with *Adh* genotype and temperature (Fig. 1e). Significant differences between *aGpdh* genotypes were found at 20°C within all *Adh* genotypes, and at 25°C only within *Adh*^{FF} and *Adh*^{FS}. At 25°C the *Adh*^{FS} genotypes did not follow the general picture in

that *aGpdh*^{FF} and *aGpdh*^{SS} were both slower than *aGpdh*^{FS}. At 29°C only within *Adh*^{FF} were significant differences found between *aGpdh* genotypes.

Adult weight

ANOVA for the effects of *Adh* genotype, *aGpdh* genotype and temperature on adult weight (Table 3) showed significant variation for all main effects, the interaction between *Adh* and *aGpdh* and the interaction between *aGpdh* and temperature. Adult weight was negatively correlated with rearing temperature (Fig. 2a). Figure 2b and c show the weight of *Adh* and *aGpdh* genotypes at 20, 25 and 29°C. *Adh*^{FS} was significantly heavier than *Adh*^{FF} and *Adh*^{SS} (Fig. 2b). No interaction was observed between the *Adh* genotype and temperature. The general picture of weight differences between *Adh* genotypes is only slightly affected by interaction with the *aGpdh* genotype (Fig. 2d). The *aGpdh* genotype interacted with temperature (Fig. 2c): at 20°C there were no significant differences, at 25°C *aGpdh*^{FF} was significantly lighter than *aGpdh*^{SS}, and at 29°C *aGpdh*^{FF} was significantly lighter than *aGpdh*^{FS}. The *aGpdh* genotypes (Fig. 2d) did not differ significantly within *Adh*^{FF} and *Adh*^{FS} genotypes but within *Adh*^{SS} genotypes *aGpdh*^{SS} was significantly heavier than *aGpdh*^{FF}, with *aGpdh*^{FS} being intermediate.

Protein content

ANOVA for the effects of *Adh* genotype, *aGpdh* genotype and temperature on protein content (Table 4) showed significant variation for *Adh* genotype and interaction between *Adh* genotype and temperature. Figure 3 shows the protein content of *Adh* genotypes

Table 2 ANOVA for developmental time (hours) of males of different *Adh* and *aGpdh* genotypes reared at different temperatures

Source	SS	d.f.	MS	F	P<
<i>Adh</i> genotype (a)	345	2	172	44	0.001
<i>aGpdh</i> genotype (g)	191	2	96	24	0.001
Temperature (t)	1145888	2	572944	145788	0.001
<i>a</i> × <i>g</i>	96	4	24	6	0.001
<i>a</i> × <i>t</i>	69	4	17	4	0.01
<i>g</i> × <i>t</i>	93	4	23	6	0.001
<i>a</i> × <i>g</i> × <i>t</i>	165	8	21	5	0.001
Explained	1146847	26	44109	11224	0.001
Residual	955	243	4		
Total	1147802	269	4267

Fig. 1 Egg-to-adult developmental time (h) of males. Vertical bars indicate 95 per cent confidence intervals. Arrows indicate significant differences with Tukey's test ($P < 0.05$). The data are standardized for temperature differences for Fig. 1b and d, these figures show the deviation from the mean of all genotypes per temperature.

Table 3 ANOVA for body weight (mg) of adult males of different *Adh* and *αGpdh* genotypes reared at different temperatures

Source	SS	d.f.	MS	F	P<
<i>Adh</i> genotype (<i>a</i>)	0.018	2	0.009	25.3	0.001
<i>αGpdh</i> genotype (<i>g</i>)	0.003	2	0.001	3.6	0.05
Temperature (<i>t</i>)	0.671	2	0.335	967.6	0.001
<i>a</i> × <i>g</i>	0.011	4	0.003	7.6	0.001
<i>a</i> × <i>t</i>	0.001	4	0.000	0.8	ns
<i>g</i> × <i>t</i>	0.006	4	0.001	4.1	0.01
<i>a</i> × <i>g</i> × <i>t</i>	0.004	8	0.001	1.5	ns
Explained	0.712	26	0.027	79.0	0.001
Residual	0.084	243	0.000		
Total	0.796	269	0.003

Fig. 2 Body weight of adult males (mg/individual). Vertical bars indicate 95 per cent confidence intervals. Arrows indicate significant differences with Tukey's test ($P < 0.05$). The data are standardized for temperature differences for Fig. 2b and d.**Table 4** ANOVA for protein content (μg protein/mg body weight) of adult males of different *Adh* and *αGpdh* genotypes reared at different temperatures

Source	SS	d.f.	MS	F	P<
<i>Adh</i> genotype (<i>a</i>)	367.0	2	183.5	19.9	0.001
<i>αGpdh</i> genotype (<i>g</i>)	27.8	2	13.9	1.5	ns
Temperature (<i>t</i>)	5.7	2	2.9	0.3	ns
<i>a</i> × <i>g</i>	39.7	4	9.9	1.1	ns
<i>a</i> × <i>t</i>	224.1	4	56.0	6.1	0.001
<i>g</i> × <i>t</i>	76.5	4	19.1	2.1	ns
<i>a</i> × <i>g</i> × <i>t</i>	107.3	8	13.4	1.5	ns
Explained	847.9	26	32.6	3.5	0.001
Residual	1698.2	184	9.2		
Total	2546.1	210	12.1		

at 20, 25 and 29°C. Combined *Adh*^{SS} had a significant lower protein content for all temperatures than *Adh*^{FF} and *Adh*^{FS} (Fig. 3a), this effect was more extreme at higher temperatures (Fig. 3b).

Triglycerides

ANOVA for the effects of *Adh* genotype, *αGpdh* genotype and temperature on adult triglycerides content (Table 5) showed significant variation for all main effects and the interaction between *Adh* and *αGpdh*. The triglyceride content was equal at 20 and 25, but significantly lower at 29°C (Fig. 4a). *Adh*^{FS} had a lower triglyceride content than *Adh*^{SS} (Fig. 4b). *αGpdh*^{FS} had a higher triglyceride content than *αGpdh*^{FF} and *αGpdh*^{SS} (Fig. 4b). Interactions between *Adh* and *αGpdh* are shown in Fig. 4c. There were no significant differences within *αGpdh*^{FF} and *αGpdh*^{FS} in the triglyceride content between the *Adh* genotypes. *Adh*^{SS} had the highest triglycerides content within *αGpdh*^{SS} genotypes. Differences between *αGpdh* genotypes vary only slightly within *Adh* genotypes.

Fig. 3 Protein content of adult males ($\mu\text{g protein mg}^{-1}$ bodyweight). Vertical bars indicate 95 per cent confidence intervals. Arrows indicate significant differences with Tukey's test ($P < 0.05$).

ADH enzyme activity

ANOVA for the effects of *Adh* and *αGpdh* genotype and temperature on ADH activity (Table 6) revealed significant variation for all three main effects and an interaction between *Adh* and *αGpdh*. ADH activity at 20°C was slightly lower than at 25 and 29°C (Fig. 5a).

Fig. 4 Triglyceride content of adult males ($\mu\text{g triglycerides mg}^{-1}$ bodyweight). Vertical bars indicate 95 per cent confidence intervals. Arrows indicate significant differences with Tukey's test ($P < 0.05$). The data are standardized for temperature differences for Fig. 4b and c

Table 5 ANOVA for triglycerides content ($\mu\text{g triglycerides/mg body weight}$) of adult males of different *Adh* and *αGpdh* genotypes reared at different temperatures

Source	SS	d.f.	MS	F	P <
<i>Adh</i> genotype (a)	1986	2	993	7.7	0.001
<i>αGpdh</i> genotype (g)	7003	2	3502	27.1	0.001
Temperature (t)	8243	2	4122	32.0	0.001
<i>a</i> × <i>g</i>	1779	4	445	3.4	0.01
<i>a</i> × <i>t</i>	1049	4	262	2.0	ns
<i>g</i> × <i>t</i>	182	4	45	0.4	ns
<i>a</i> × <i>g</i> × <i>t</i>	685	8	86	0.7	ns
Explained	21057	26	810	6.3	0.001
Residual	23735	184	129		
Total	44791	210	213		

Table 6 ANOVA for ADH enzyme activity (nmol NADH min⁻¹ µg⁻¹ protein) of adult males of different *Adh* and *αGpdh* genotypes reared at different temperatures

Source	SS	d.f.	MS	F	P<
<i>Adh</i> genotype (<i>a</i>)	14.98	2	7.490	2223.5	0.001
<i>αGpdh</i> genotype (<i>g</i>)	0.38	2	0.188	55.8	0.001
Temperature (<i>t</i>)	0.06	2	0.031	9.2	0.001
<i>a</i> × <i>g</i>	0.15	4	0.038	11.3	0.001
<i>a</i> × <i>t</i>	0.03	4	0.008	2.4	ns
<i>g</i> × <i>t</i>	0.01	4	0.001	0.3	ns
<i>a</i> × <i>g</i> × <i>t</i>	0.4	8	0.004	1.3	ns
Explained	15.51	26	0.597	177.1	0.001
Residual	0.58	173	0.003		
Total	16.10	199	0.081

Fig. 5 ADH enzyme activity of adult males (nmol NADH min⁻¹ µg⁻¹ protein). Vertical bars indicate 95 per cent confidence intervals. Arrows indicate significant differences with Tukey's test ($P < 0.05$). The data are standardized for *Adh* genotype differences for Fig. 5a

The interaction between *Adh* and *αGpdh* is shown in Fig. 5b. The ADH activity of *Adh*^{FF} was higher than *Adh*^{SS} within all *αGpdh* genotypes, with *Adh*^{FS} being intermediate. There were significant differences

between *αGpdh* genotypes within *Adh*^{FF} and *Adh*^{FS} genotypes: *αGpdh*^{FF} had higher ADH activity than *αGpdh*^{SS}, with *αGpdh*^{FS} being intermediate. The differences in ADH enzyme activity of identical *Adh* genotypes between *αGpdh* genotypes were small compared to differences between *Adh* genotypes.

αGPDH enzyme activity

ANOVA for the effects of *Adh* and *αGpdh* genotype and temperature on *αGPDH* enzyme activity (Table 7) revealed a significant variation for temperature, *Adh* genotype and the interaction between *Adh* and *αGpdh*. *αGPDH* activity at 29°C was slightly lower than at 25 and 20°C (Fig. 6a). An interaction between the *Adh* and *αGpdh* genotypes is shown in Fig. 6b. *Adh*^{SS}*αGpdh*^{FF} had significantly higher activity than *Adh*^{SS}*αGpdh*^{FS} and *Adh*^{SS}*αGpdh*^{SS}. The differences in *αGPDH* enzyme activity were very small compared to the differences in ADH enzyme activity.

Correlation between characters

Table 8 shows the correlations between the studied traits, calculated from genotype means standardized for temperature differences. Two trends can be derived from these data.

1 There is a significant positive correlation between weight, protein content (per individual) and *αGPDH* enzyme activity (per individual). These variables are not correlated if protein content is calculated per milligram of bodyweight and the *αGPDH* enzyme activity is calculated per microgram of protein (Table 8, below diagonal), so these correlations may be an effect of size: heavier genotypes have proportionally more protein and *αGPDH* enzyme activity.

2 There is a significant negative correlation between developmental time on the one hand and ADH and

Table 7 ANOVA for α GPDH enzyme activity ($\text{nmol NADH min}^{-1} \mu\text{g}^{-1}$ protein) of adult males of different *Adh* and *αGpdh* genotypes reared at different temperatures

Source	SS	d.f.	MS	F	P<
<i>Adh</i> genotype (<i>a</i>)	0.013	2	0.006	3.2	0.05
<i>αGpdh</i> genotype (<i>g</i>)	0.009	2	0.005	2.3	ns
Temperature (<i>t</i>)	0.027	2	0.014	6.9	0.001
<i>a</i> × <i>g</i>	0.077	4	0.019	9.8	0.001
<i>a</i> × <i>t</i>	0.017	4	0.004	2.1	ns
<i>g</i> × <i>t</i>	0.005	4	0.001	0.7	ns
<i>a</i> × <i>g</i> × <i>t</i>	0.020	8	0.003	1.3	ns
Explained	0.169	26	0.006	3.3	0.001
Residual	0.360	183	0.002		
Total	0.528	209	0.003

Fig. 6 α GPDH enzyme activity of adult males ($\text{nmol NADH min}^{-1} \mu\text{g}^{-1}$ protein). Vertical bars indicate 95 per cent confidence intervals. Arrows indicate significant differences with Tukey's test ($P < 0.05$). The data are standardized for temperature differences for Fig. 6b.

α GPDH enzyme activity on the other. The same trend, although not significant, is found for protein and weight in relation to developmental time. We therefore found no trade-off between developmental time and these traits. Triglyceride content, however, does not follow this trend, and is not correlated with developmental time or weight.

ADH enzyme activity is significantly negatively correlated with developmental time. Combining this with the data in Figs 1b and 5b, it can be concluded that the negative correlation between ADH enzyme activity and developmental time coincides with *Adh* genotype. Within the *Adh* genotypes, however, the correlation between ADH enzyme activity and developmental time becomes positive, taken the data standardized for *Adh* genotype differences (Table 8, ADHst). Combining this with the data in Figs 1b and 5b, it appears that the positive correlation between ADH enzyme activity and developmental time within *Adh* genotypes and temperatures coincides with the *αGpdh* genotype: *αGpdh*^{FF} have higher ADH enzyme activity and longer developmental time than *αGpdh*^{SS} genotypes, with *αGpdh*^{FS} genotypes being intermediate.

Discussion

The developmental rate was affected by both the *Adh* and the *αGpdh* genotype. *Adh*^{FF} developed faster than *Adh*^{SS}, and *Adh*^{FS} was intermediate. Literature on the developmental rate of *Adh* genotypes comes from (i) selection experiments for developmental rate in polymorphic populations (Van Delden & Kamping, 1979; Cavener, 1983; Knibb *et al.*, 1987), (ii) determination of genotype frequencies in fast and slow developing groups within polymorphic populations (Van Delden & Kamping, 1979; Marinković *et al.*, 1987), and (iii) measurement of developmental rate of homozygous strains (Kerver & Van Delden, 1985; Kohane & Parsons, 1986; Izquierdo & Rubio, 1989). Although some of these studies revealed no differences in the rate of development between *Adh* genotypes, most studies indicated that *Adh*^{FF} develops faster than

Table 8 Coefficients of correlation (r) between developmental time, adult body weight, protein content and triglycerides content and ADH and α GPDH enzyme activity, calculated from genotype means ($n = 27$) standardized for temperature differences. Above the diagonal protein, triglycerides, ADH and α GPDH are expressed per individual, below the diagonal protein and triglycerides are expressed per milligram of bodyweight, and ADH and α GPDH per microgram of protein. For the variable ADHst data are standardized for temperature and *Adh* genotype differences

	Developmental time	Weight	Protein	Triglyceride	α GPDH	ADH	ADHst
Developmental time		-0.21	-0.32	0.00	-0.43*	-0.52**	0.41*
Weight	-0.21		0.62**	0.14	0.56**	0.16	0.23
Protein	-0.27	0.27		0.02	0.79**	0.57**	0.08
Triglyceride	0.05	-0.05	-0.11		-0.27	-0.10	0.22
α GPDH	-0.24	-0.03	-0.26	-0.47*		0.58**	-0.15
ADH	-0.52**	0.14	0.61**	-0.14	0.12		
ADHst	0.44*	0.13	-0.04	0.13	-0.14		

*Significant at 0.05 level.

**Significant at 0.01 level.

Adh^{SS}, with *Adh*^{FS} being intermediate or equal to one of the homozygous genotypes. Our results are thus well in agreement with these findings. Kohane & Parsons (1986) found no interaction between the rate of development of *Adh* genotypes and temperature.

α Gpdh^{FF} developed more slowly than *α Gpdh*^{FS} and *α Gpdh*^{SS}. The literature on developmental rate differences between *α Gpdh* genotypes is ambiguous. Selection for developmental rate (Cavener, 1983) indicated a relative faster development of *α Gpdh*^{FF}. The determination of genotype frequencies in fast and slow developing groups of a polymorphic population (Marinković *et al.*, 1987) also indicated a relative faster development of *α Gpdh*^{FF}. Measurement of the developmental rate of homozygous strains by Izquierdo & Rubio (1989), however, showed a relative faster development of *α Gpdh*^{SS}. Measurement of the developmental rate at different temperatures (Barnes *et al.*, 1989) indicated a relative faster developmental rate of *α Gpdh*^{SS} at low temperature. So the data on homozygous strains are in agreement with our data, in contrast to the data on polymorphic populations. This contradiction may be explained by density-dependent effects. Both experiments with polymorphic populations (Cavener, 1983; Marinković *et al.*, 1987) were performed under high-density conditions, as can be concluded from the fact that in both experiments adult eclosion was spread over at least 10 days. Measurements on homozygous strains (Izquierdo & Rubio, 1989; Barnes *et al.*, 1989; our paper) were performed at rather low densities. So *α Gpdh*^{SS} may have a relative faster development at low and *α Gpdh*^{FF} at high densities. Density-dependent effects for the viability of *α Gpdh* genotypes are known from the experiments of Charles-Palabost (1982) who found

higher viability of the *α Gpdh*^{FF} genotypes than *α Gpdh*^{SS} genotypes under low density conditions, but a reversal at high density.

The interaction between the effects of *α Gpdh* genotypes and rearing temperature on developmental rate (*α Gpdh*^{FF} genotypes developed more slowly than *α Gpdh*^{FS} and *α Gpdh*^{SS} genotypes at 20 and 25, but not at 29°C) is in agreement with the data of Barnes *et al.* (1989). Interaction between the effects of *Adh* and *α Gpdh* genotype on developmental time (Fig. 1d) chiefly concerned the relative position of the heterozygous genotypes, in some cases they were equal to the one homozygous genotype, in other cases they were equal to the other homozygous genotype. The interaction between the *Adh* and *α Gpdh* genotype and temperature (Fig. 1e) is very interesting. Significant differences between *α Gpdh* genotypes were found at 20°C within all *Adh* genotypes, at 25°C only within *Adh*^{FF} and *Adh*^{FS}, and at 29°C only within *Adh*^{FF}.

Is the correlation between developmental rate and *Adh* and *α Gpdh* genotype caused by differences in metabolic flux through pathways in which ADH and α GPDH are involved? Allozymes can influence metabolic processes by differences in the amount or activity. We found that *Adh*^{FF} strains had a 3.5 time higher ADH activity than *Adh*^{SS} strains. This difference is well known from the literature (see review by Chambers, 1988): in *Drosophila melanogaster* strains that carry the *Adh*^S allele generally have lower ADH activity levels than lines with *Adh*^F alleles, which is largely accounted for by differences in protein level. We found no differences in α GPDH enzyme activity between *α Gpdh* genotypes. This is in agreement with the data of Laurie-Ahlberg (1985), who found that over a large number of strains the α GPDH enzyme

activity of *αGpdh*^{SS} genotypes on average was slightly higher than that of *αGpdh*^{FF} strains, although there was a very broad overlap. We found only small interactions between the *Adh* and *αGpdh* genotype for both ADH (Fig. 5b) and *α*GPDH (Fig. 6b) enzyme activity. As *in vivo* substrate concentrations are generally too low to saturate enzymes, $V_{\max}$ (essentially the enzyme activity as measured here) is not an appropriate description of *in vivo* enzyme activity, instead $V_{\max}/K_m$ may be a better approximation (e.g. Middleton & Kacser, 1983). Such calculations for ADH still result in a higher enzyme activity of *Adh*^{FF} compared with *Adh*^{SS}, independent from the temperature (Alahiotis, 1982; McElfresh & McDonald, 1986; Heinstra *et al.*, 1988). The enzyme activity differences for *α*GPDH between the allozymes are not as clear as for ADH, but a strong temperature effect is described (Miller *et al.*, 1975; Alahiotis *et al.*, 1977; McKechnie *et al.*, 1981; not found by Bewley *et al.*, 1984): the K_m (DHAP) between 20 and 30°C is constant for *αGpdh*^{FF} but increases with temperature for *αGpdh*^{SS} and *αGpdh*^{FS}. If we assume no substantial relative differences in $V_{\max}$, the *in vivo* enzyme activity of *αGpdh*^{FS} and *αGpdh*^{SS} might decrease, compared to *αGpdh*^{FF}, with increasing temperature. This relative increase of *α*GPDH *in vivo* enzyme activity of *αGpdh*^{FF} is correlated with our finding of a relative increase in the developmental rate of *αGpdh*^{FF} with increasing temperature (Fig. 1c). This might indicate a positive correlation between *α*GPDH enzyme activity and the developmental rate.

It has to be stressed that the observed developmental rate differences between genotypes are rather small: a maximum of 2 per cent. A selection experiment in our laboratory (B. J. Zwaan, in preparation) for fast and slow development in the same strain, Groningen 83, revealed, after 10 generations of selection, a difference of 15 per cent between the fast and the slow selection lines. The relative small effect of *Adh* and *αGpdh* is in agreement with the metabolic control theory (e.g. Hartl & Clark, 1989) that predicts that metabolic flux differences, due to activity differences between allozymes, are very small because enzymes are embedded in a matrix of many other enzymes. Indeed, the metabolic pathways in which ADH and *α*GPDH function are rather diffuse. Geer *et al.* (1985) found that ADH plays a role in the conversion of both ethanol and sucrose to lipids (fatty acids) in larvae of *Drosophila melanogaster*. Heinstra *et al.* (1990) showed that carbon atoms from ethanol are also incorporated in carbohydrates and amino acids. The oxidation of ethanol by ADH generates NADH. *α*GPDH plays an important role in the flight metabolism of adult *Drosophila melanogaster* in regenerating NAD⁺ in concert with the mitochondrial *α*GPO

forming the '*α*GPDH-shuttle' (O'Brien and MacIntyre, 1978). An analogous role of *α*GPDH in the regulation of NAD⁺/NADH ratios in larval tissues is proposed by several authors (e.g. Rechsteiner, 1970; Geer *et al.*, 1983). Further *α*GPDH converts dihydroxyacetone phosphate to glycerophosphate, a precursor for phospholipids and triglycerides (O'Brien and MacIntyre, 1978; Geer *et al.*, 1983).

Heterozygote superiority was found for adult body weight (*Adh*^{FS} genotypes were the heaviest) and triglyceride content (*αGpdh*^{FS} genotypes had the most triglycerides). Partridge (1988) found a correlation between body size and lifetime reproductive success in both males and females. Lipid content is related to resistance to starvation (David *et al.*, 1983; Zwaan *et al.*, 1991) and viability and fecundity (Clark, 1989). As heterozygote effects are supposed to play an important role in the maintenance of the polymorphism of both *Adh* and *αGpdh* in nature (Bijlsma-Meeles & Bijlsma, 1988; Chambers, 1988), our data fit well. We have planned further research on these traits.

The adaptive consequences of the developmental rate are rather complex. A fast rate of development generally lead to a higher fitness. Parsons (1983), however, pointed out that there might be a difference between climates. In extreme climates (far from the equator) there is an advantage for the *r*-strategy, with a fast rate of development. Under more optimal conditions (near the equator) there is an advantage for the *K*-strategy, with a slow rate of development. In the light of this theory, our data on the developmental time of *Adh* and *αGpdh* genotypes fit well with the world-wide cline of *Adh* and *αGpdh* allele frequencies. *Adh*^S and *αGpdh*^F frequencies are high near the equator (Oakeshott *et al.*, 1982) and we found that these alleles are related to slow development, which provides an advantage in these regions. At higher latitudes *Adh*^F and *αGpdh*^S are more frequent (Oakeshott *et al.*, 1982) and we found that these alleles are related to fast development, which also provides an advantage in these regions. Our data thus indicate that the developmental rate differences between the genotypes might be one of the underlying mechanisms for the allele frequency clines.

Furthermore, this study has shown that several differences occur among both *Adh* and *αGpdh* genotypes for traits which may have adaptive consequences. In addition, an interaction between *Adh* and *αGpdh* may occur. The differences observed, except ADH enzyme activity between *Adh* genotypes, are generally very small; it is conceivable that under more stressful conditions than those applied in these experiments, these differences might be emphasized.

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